LXXXIX. CARBOHYDRATE AND FAT METABOLISM IN YEAST.

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PART I. THE FORMATION OF FAT FROM NON-NITROGENOUS SUBSTANCES OTHER THAN SUGARS.

Forty-five years ago Nägeli [1878, 1879] examined the production of fat by moulds and yeasts and found that the fat content of these organisms could be increased from 5 % to 12 % when they were grown in a well-aerated medium rich in carbohydrate and poor in nitrogen. During the war Lindner [1922] and his co-workers applied similar conditions of growth to the organism *Endomyces vernalis*, and the fat content of this was so successfully raised that it was used in Germany to provide rations both of fat and protein. Lindner also made a histological investigation of yeast cells grown under similar conditions of aeration in a medium rich in carbohydrate and poor in nitrogen. He describes as the first stage of fat formation a granular appearance of the plasma, the granules coalescing gradually to form fat globules, which could be stained with appropriate reagents, *e.g.* osmic acid, Sudan III or alkanna. Solutions of sugar and of alcohol were found to be suitable media from which the yeast cell could increase its quantity of stored fat. Superficial cultures of yeast cells were also found to become laden with fat globules when submitted to the action of alcohol vapour. Berzfeld [1922] described the power of the yeast cells to stain with alkanna and Sudan III as a criterion of age, quality and degeneration and found it was not till the third or fourth generation that the fat in the yeast cell could be shown by staining. There are also a number of patents dealing with the changes produced by different media on the fat content of yeast. Quantitative data have been however almost entirely lacking and even the relative efficiency of sugar and alcohol as sources from which the yeast cell could produce fat does not seem to have been determined.

Nägeli was aware that the direct extraction of dried yeast by ether did not remove all the fat it contained, but subsequent workers do not appear to have recognised this. In an earlier communication by one of us it was shown that the amount of fat which could be obtained from a sample of yeast depended on the method of extraction used [Smedley MacLean, 1922]. The proportion of the total fat present which could be extracted directly from dried yeast in a Soxhlet apparatus by ether also varied according to the previous history of the yeast. From yeast previously incubated in a well-oxygenated solution of sugar only a small proportion of the fat it contained was separated by direct extraction, but if the yeast was first hydrolysed by boiling it for two

hours with normal acid and the residue dried and extracted with ether a much larger quantity of fat was obtained than by the direct extraction process; the acid value of the fat was only slightly raised, showing that hydrolysis had only taken place to a very small extent. If the fat was estimated in an old sample of yeast both by ether extraction of the dried yeast and by extraction of the dried residue after hydrolysis there was much less difference between the results obtained by the two methods than in the case of the recently incubated yeast described above. The fat content of all samples of yeast was however found to be higher when determined after hydrolysis of the yeast.

The hydrolysis method has also the advantage of allowing the carbohydrate content of the yeast to be determined at the same time in the filtrate so that the relation between the fat and carbohydrate contents of the yeast may be conveniently studied. By the method of acid hydrolysis any lipin present in the fat would be partly decomposed and glycerophosphoric acid separated so that if any lipin were present the fat obtained by direct extraction would lose a fraction of its weight during the hydrolysis.

It is clear that care must always be taken to differentiate between a real and an apparent increase of fat in the cell. Since the fat when first formed in the yeast cell appears to be in a condition in which it cannot be directly extracted by ether, the effect of incubating the yeast in a certain medium might conceivably be to produce an apparent increase of fat by altering the state in which the fat is held in the cell and rendering it capable of being extracted by solvents. If the yeast be first heated with dilute acid as described this difficulty is eliminated.

It seemed to us that it would be of interest to obtain quantitative data as to the amount of fat stored in yeast when the yeast is incubated in solutions of various non-nitrogenous organic substances and we investigated therefore the action in this respect of the following:

(1) simple organic substances such as alcohols and the sodium salts of fatty acids;

(2) the sugars: lactose, glucose, fructose, sucrose and maltose;

(3) sugar solutions with the addition of alkali phosphates.

Method of experiment.

Samples of the yeast were weighed out for the determination of moisture and nitrogen and four quantities each of 12·5 g. were also weighed; two of these samples were hydrolysed by boiling each with 100 cc. of *N* HCl for two hours under a reflux condenser; the mixture was filtered and the residue well washed with hot water; to the filtrate and washings 80 cc. of *N* NaOH were added and about 30 cc. of "dialysed iron" and the mixture was made up to 1 litre. After filtering, the reducing power of the filtrate was determined by Bertrand's method and the total reducing carbohydrate present after hydro-

lysis calculated as glucose for the purpose of comparison. The residue of yeast cells after boiling with the acid was air dried over-night on the filter and then extracted with ether for 48 hours in a Soxhlet apparatus. After evaporation of the ether the residue was taken up with dry ether, filtered into a weighed flask, the ether evaporated and finally the ether-soluble substance dried to constant weight in a vacuum desiccator. The two remaining quantities of 12·5 g. of yeast were added each to a litre of the solution to be tested contained in a Winchester quart bottle. Into one of these a tube was fitted through the cotton-wool plug and after placing in the incubator at 25–26°, a current of oxygen was passed through for 45 hours; the other was placed in the incubator, no oxygen being passed through. At the end of the experiment the contents of each were centrifuged and the yeast filtered on a Buchner funnel, well washed and weighed. Samples were taken for the determination of moisture and nitrogen and the remainder hydrolysed and treated exactly as described for the original yeast.

The substance soluble in the dry ether was regarded as fat; the acid value of the fat obtained in this way was found to be slightly higher than that of the fat directly extracted by ether from the dried yeast. It is possible that the slight increase is due to acid liberated from the lipins present. The yeast sterol, previously identified as ergosterol [Smedley MacLean and Thomas, 1920], is always present with the fat. In our earlier experiments the pressed yeast used was supplied by a yeast dealer, but the results obtained were not consistent; the age of the yeast seemed to be an important factor since the power of a specimen of pressed yeast to form fat fell off rapidly when the yeast was kept in the cold room for one or two days before being used. We subsequently worked with an ale yeast from 84 to 110 hours old supplied directly from a brewery where we could rely on the yeast being grown under approximately constant conditions; this yeast we found contained appreciably less sterol than that used in our earlier experiments.

The effect on the fat content of yeast produced by incubating it in solutions of simple organic substances.

The effect of incubating yeast for 45 *hours in water at* 25° *with and without oxygenation* was first ascertained as a basis of comparison for future experiments. We found that 12·5 g. of brewery yeast incubated for 45 hours at 25° in a litre of water and oxygenated throughout this period lost about two-thirds of the carbohydrate originally present and about a seventh of the protein; the quantity of fat however increased by from 50 to 100 % of that originally present. If the oxygen was not passed there was slightly less loss of carbohydrate and of protein and no increase of fat. These experiments confirm the results previously arrived at [Smedley MacLean, 1922] that a yeast with a high carbohydrate content loses carbohydrate and gains fat when incubated in oxygenated water. The specimens of pressed yeast ex-

amined at an earlier date had been kept for some time and did not show the increase of fat after incubation in water, but as stated above we found that the fat-forming power of a yeast rapidly fell off on keeping the yeast in the cold room.

In subsequent experiments carried out with 12·5 g. of yeast containing approximately 0·1 g. of fat we have regarded increases of fat up to 0·1 g. as capable of being formed from materials originally present in the cell since this increase could be obtained by incubating the yeast in oxygenated water. If the amount of fat was increased beyond this we have attributed it to the influence of the substance dissolved in the medium.

The substances investigated were the alcohols, methyl, ethyl, propyl, *iso*-propyl, butyl and *iso*-amyl, glycol and glycerol; acetone, acetaldehyde and the sodium salts of the following acids, formic, acetic, butyric, lactic and pyruvic. The method of procedure was to make up a solution of the substance to be tested, generally of decinormal strength and to incubate the yeast in this as described above.

We found we could divide these substances into three classes:

(*a*) *Those producing a definitely inhibitory effect*, no increase of fat being observed or an amount so small as to be within the region of experimental error. Under this heading we include methyl, propyl, butyl and *iso*-amyl alcohols, that is to say that all the normal fatty alcohols tested, with the exception of ethyl alcohol, appear to be definitely injurious to the formation of fat when supplied in decinormal solution, less fat being found than when the yeast was incubated under similar conditions in water. We found also that acetaldehyde supplied in 0·6 % concentration produced little change in the fat and carbohydrate content; it appeared to be injurious to the yeast and there was a marked increase in the loss of protein. These results contrast with those obtained by Haehn [1923] in his recently published paper on *Endomyces vernalis*, where a marked increase of fat was observed when the organism was supplied with acetaldehyde in a concentration of 1 %, alkali phosphates being also present in the solution.

(*b*) *Substances, solutions of which behave similarly to water* and which may therefore be assumed to have no influence on the production of fat. Decinormal solutions of the sodium salts of formic, propionic and butyric acids, of *iso*-propyl alcohol and acetone, glycol in 0·5 % and glycerol in both 0·5 and 5 % solutions belong to this class. Small increases of fat were recorded quite comparable with those observed when the yeast was incubated in water and the amount of carbohydrate found at the end of the period of incubation was also of the same order in both cases.

(*c*) *Substances which produce an increase of fat greater than that observed after incubation of yeast in water.* To this class belong ethyl alcohol, and the sodium salts of acetic, pyruvic and lactic acids.

Ethyl alcohol. Our experiments showed that if yeast were incubated for 45 hours in a 0·5 or 0·6 % solution of ethyl alcohol, the increase of fat in the

yeast at the end of the incubation period was more than after incubation in water for the same time; in one experiment only did we get an increase of fat comparable with that obtained after yeast had been incubated in a sugar solution, but in six other experiments carried out under as far as we knew similar conditions the increase was very much smaller. If we increased the percentage of alcohol in the medium, the amount of fat stored showed no corresponding increase but a diminution.

Lindner and Unger [1919] have described the effect of alcohol vapour on different brewery yeasts and have shown that it brings about the deposition of globules of oil in the cell. It is possible that this result may be partly due to a change in the state of fat in the cell whereby the separation of the fat already present in the cell is affected. Also, that the microscopic changes which Lindner himself has described, the preliminary granulation of the cell plasma gradually followed by the collection of the granules in globules, may be hastened by the effect of the alcohol vapour. Lindner [1922] found that both with brewery yeasts and with *Endomyces vernalis* the assimilation of alcohol leads to the production of fat. In our experiments with brewery yeast alcohol is very much less effective as an aid to the formation of fat than any of the fermentable sugars. In 0·5 % solution, ethyl alcohol is as effective as a 0·5 % sugar solution in producing fat, but the amount of fat formed does not increase when the concentration of alcohol in the solution is increased and alcohol cannot therefore be put into the same category as the sugars when classified according to its fat-forming power.

Sodium acetate we found rather more effective as a fat former than ethyl alcohol, but again when the concentration of the acetate was increased above decinormal there was a diminished increase of fat. The same held also for sodium lactate and for sodium pyruvate.

These four substances can certainly be classed together as having a distinct though small influence on the production of fat. We do not however feel justified in saying that from these experiments there is any satisfactory evidence that any one of these substances can be regarded as an intermediate stage in the process of fat formation.

The increase of fat observed after incubating yeast in oxygenated water clearly proves that fat is formed from the carbohydrate held in the cell, since the loss of protein is insufficient to account for it. It may be that by the assimilation of such substances as ethyl alcohol or sodium acetate the carbohydrate storage is directly influenced and the fat formation is only indirectly influenced, and it is interesting to note that in these cases the carbohydrate content of the yeast is higher after incubation in the oxygenated than in the non-aerated solution and the amount stored may even exceed that present in the original sample of yeast. Except for one experiment with alcohol in which an abnormally high fat content was obtained and which we have never been able to repeat, the largest increase of fat was obtained after incubation in oxygenated sodium acetate solution where we got an increase of from 0·1

Table I.

Medium	Original yeast: Age of yeast. Hours	Original yeast: Carbohydrate g.	Original yeast: Fat g.	Original yeast: Protein g.	Oxygenated yeast: Carbohydrate g.	Oxygenated yeast: Fat g.	Oxygenated yeast: Protein g.	Non-oxygenated yeast: Carbohydrate g.	Non-oxygenated yeast: Fat g.	Non-oxygenated yeast: Protein g.
Water	88	1·21	0·0897	1·31	0·39	0·1420	1·12	0·50	0·0912	1·11
	110	0·75	0·1042	1·20	0·30	0·1590	1·02	0·31	0·0932	1·08
	70	1·52	0·1039	1·15	0·57	0·1978	0·98	0·65	0·1051	1·05
Substances, incubation in solutions of which produces less increase of fat than incubation in water at 25°.										
Alcohols:										
Methyl *M*/10	106	0·64	0·1229	1·29	0·27	0·1463	1·26	0·33	0·1200	—
Propyl *M*/10	83	0·72	0·1027	1·25	0·30	0·1159	1·08	0·37	0·1049	1·14
Butyl *M*/10	102	0·70	0·0817	1·40	0·32	0·0898	1·27	0·31	0·0784	1·18
iso-Amyl *M*/10	108	0·65	0·1107	1·37	0·28	0·1096	1·18	0·31	0·1127	1·18
Acetaldehyde:										
0·1 %	108	0·91	0·1050	1·46	0·37	0·1500	1·33	0·53	0·1110	1·41
0·6	78	0·99	0·0978	1·26	0·92	0·0890	0·82	0·87	0·0906	0·75
Substances, solutions of which act like water.										
Na formate *M*/10	86	1·52	0·0918	1·29	0·56	0·1841	1·17	0·46	0·0935	1·30
Na propionate *M*/10	62	1·20	0·1176	1·22	0·32	0·1856	1·10	0·36	0·1150	1·09
Na butyrate *M*/10	—	0·47	0·0981	1·29	0·27	0·1849	1·15	0·37	0·1187	1·17
	84	1·39	0·0920	1·30	0·70	0·1625	1·20	0·74	0·1033	1·24
iso-Propyl alcohol *M*/10	106	0·98	0·0893	1·32	0·38	0·1745	1·01	0·37	0·0968	1·20
Acetone *M*/10	85	1·22	0·1006	1·43	0·57	0·1667	1·26	0·65	0·1040	1·33
Glycol 0·5 %	85	1·29	0·0920	1·35	0·39	0·1497	1·21	0·41	0·0816	1·20
„ 0·5	101	0·74	0·1092	1·44	0·36	0·1404	1·29	0·42	0·1010	1·34
Glycerol 0·5 %	84	1·15	0·1228	1·20	0·46	0·2086	1·10	0·47	0·1073	1·18
„ 5·0	72	1·19	0·1167	1·23	0·43	0·1997	1·11	0·33	0·1148	1·13
Substances, solutions of which produce marked increase of fat.										
Ethyl alcohol:										
0·5 %	103	1·27	0·0862	1·08	0·53	0·2610	1·24	0·46	0·1032	1·22
0·5	83	0·83	0·1065	1·36	0·50	0·2417	1·26	0·44	0·1144	1·22
0·5	86	0·89	0·1065	1·24	0·71	0·5221	1·16	0·54	0·0984	1·20
0·5	83	1·12	0·1163	1·23	0·66	0·1889	1·19	0·74	0·1254	1·20
0·64	130	1·03	0·1110	1·34	0·43	0·2636	1·22	0·38	0·1125	1·22
2·0	106	0·97	0·1122	1·34	0·41	0·2225	1·24	0·33	0·1064	1·21
4·0	76	1·30	0·1140	1·24	0·47	0·1952	1·09	0·47	0·1032	1·15
Sodium acetate:										
0·6 %	—	0·51	0·1816	1·48	1·01	0·3930	1·35	0·40	0·1882	1·45
	96	0·60	0·1084	1·33	0·34	0·3475	1·16	0·22	0·1062	1·19
	84	1·21	0·0846	1·32	1·10	0·2136	1·22	0·50	0·0897	1·21
1·0	83	1·12	0·1034	1·23	0·41	0·2182	1·11	0·43	0·1059	1·35
2·0	106	1·14	0·0927	1·36	0·34	0·2172	1·25	0·42	0·0945	1·26
5·0	105	0·80	0·1126	1·27	0·28	0·1417	0·93	0·26	0·1049	0·86
Sodium lactate:										
0·90 %	82	1·10	0·1208	1·31	0·42	0·2470	1·23	0·30	0·1180	1·09
	107	0·80	0·1058	1·31	0·39	0·1664	1·15	0·37	0·1080	1·08
2·0	84	1·31	0·1035	1·23	0·51	0·2113	1·16	0·43	0·1070	1·20
Sodium pyruvate:										
0·88 %	82	1·31	0·0945	1·29	0·53	0·2291	1·19	—	—	—
	94	0·89	0·1026	1·39	0·47	0·2204	1·20	—	—	—
	96	1·14	0·1006	1·33	0·60	0·2461	1·32	0·69	0·1185	1·33
	81	1·05	0·0932	1·31	0·40	0·2270	1·24	0·38	0·1153	1·17

to 0·35 g., an increase of about 10 % on the dry weight of the yeast. Since in some of the acetate experiments there was at the end of the experiment an actual increase in the amount of the carbohydrate stored in the cell, it

follows that the cell was able to build up carbohydrate from the acetate supplied and some of the newly formed carbohydrate may have been converted to fat.

It may be that we cannot present any simple intermediate substance to the cell under suitable conditions for the cell to make use of it; it does not necessarily follow that these substances may not occur in the cell as intermediate substances. It is interesting to note that Stephenson [1922] found that sodium acetate exerted a beneficial effect on fat formation in the Timothy Grass Bacillus.

PART II. THE RELATIVE EFFICIENCY OF THE DIFFERENT SUGARS IN PRODUCING STORAGE OF CARBOHYDRATE AND OF FAT.

When yeast is incubated in an oxygenated solution of fermentable sugar, the fat content is increased.

In stating our results we have recorded the actual amounts of fat, carbohydrate and protein in the yeast before incubation and compared these with the amounts present when the same weight of the yeast has been incubated in a solution of the substance under investigation. A comparison of the percentages of fat and carbohydrate found in the samples of yeast before and after incubation calculated on the dry weight of the yeast may give rise to misleading conclusions. No nitrogen being present in the nutrient solutions, an increase in weight must be due to an increase in the amount of fat or carbohydrate present. If the latter be increased, although no fat may have been lost, the percentage of fat will be diminished and similarly a diminution of the carbohydrate content produces an apparent increase of fat when expressed as fat percentage although the total amount of fat present may be less than at the beginning of the experiment.

The method of carrying out the experiments was the same as that described above for the simpler carbon compounds (cp. p. 721). The pressed yeast with which we worked showed comparatively small variations in the percentage of fat it contained. The 12·5 g. used for each experiment contained approximately 0·1 g. fat, the amount in the vast majority of samples lying between 0·08 and 0·1 g. and the results of the experiments were practically unaffected by these small differences.

The variations in the carbohydrate contents of the original pressed yeast were very much wider and seem to influence to some extent the final figures for the carbohydrate when the yeast is incubated in a 0·5 or 1·0 % solution of sugar. In stronger solutions the influence does not seem to be perceptible. A survey of the accompanying tables will however show that the amounts of carbohydrate contained in a fixed weight of yeast after it has been incubated in a sugar solution are remarkably constant for any given concentration of solution and must depend mainly on this factor.

With regard to the nature of the fat formed it appears to be closely similar to that present in the original yeast; it shows the same power of remaining

concealed in the cell until after the yeast has been hydrolysed with dilute acid, which has already been described. The saponification number of the fat from different samples lay between 139 and 186 and the Hübl Iodine Values varied considerably, the limits being from approximately 80 to 132. These differences may be partly explained by variations in the amount of ergosterol present and partly perhaps by oxidation, as the newly formed fat

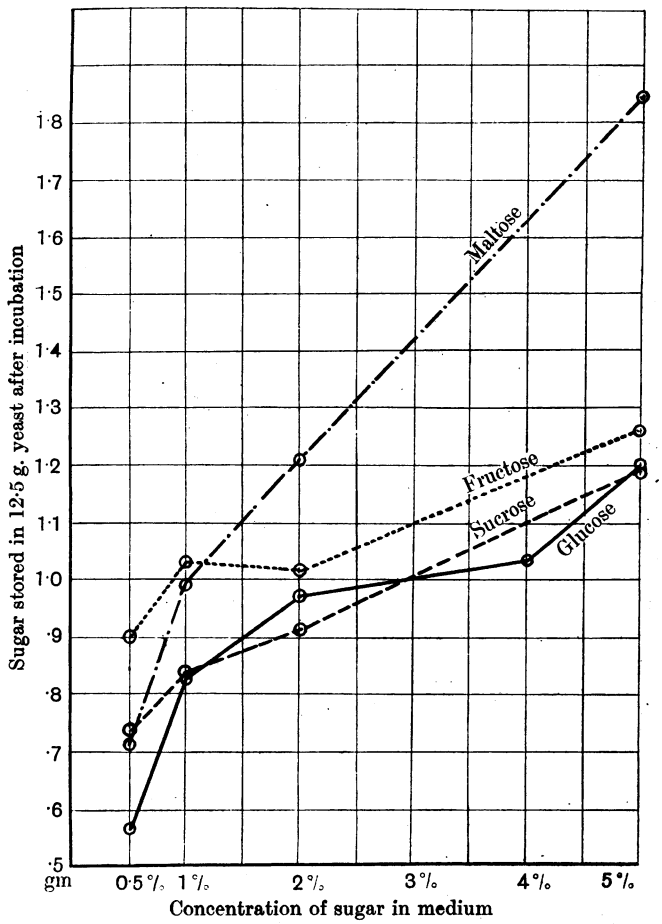

Fig. 1. Curves showing relation of concentration of sugar in oxygenated medium to amount of carbohydrate stored by yeast.

seemed very susceptible to oxidation. We were unable to detect any consistent differences between the fat formed under different conditions and have therefore not recorded these figures in detail.

The sugars investigated were lactose, glucose, fructose, sucrose and maltose.

Lactose. Of these the only sugar which was not fermented by the yeast was lactose and it alone appeared to exercise no influence on the storage of either carbohydrate or fat within the yeast cell. A 5 % solution of lactose

behaved very much like water; yeast when incubated in it for 45 hours at 25° showed a small storage of fat and a considerable loss of carbohydrate if the solution had been oxygenated and no storage of fat and rather less loss of carbohydrate in the yeast from the non-oxygenated medium.

Glucose, fructose and sucrose. These three sugars closely resembled each other in their influence on the storage of both carbohydrate and fat. After

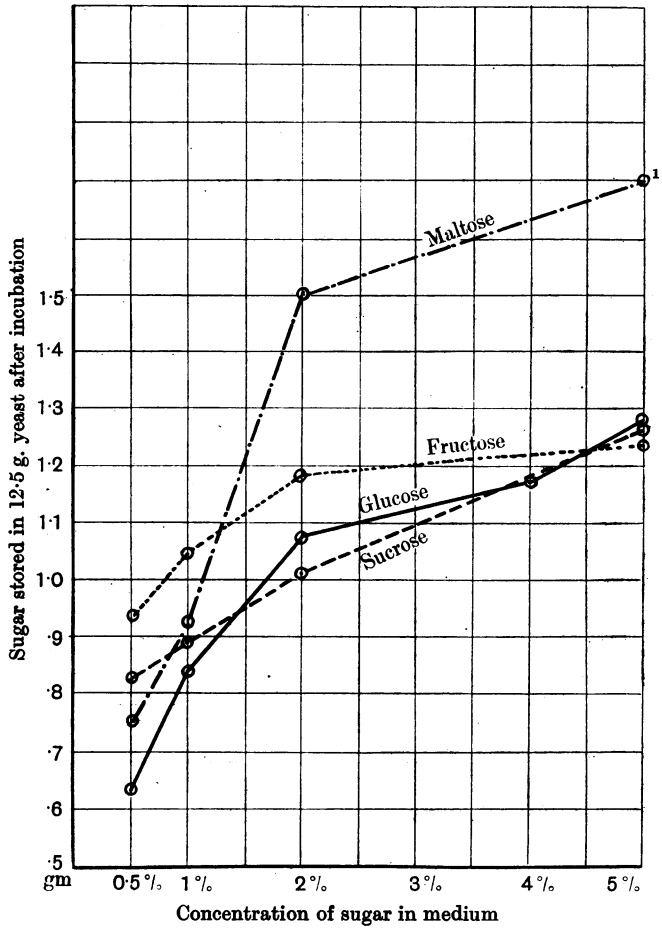

Fig. 2. Curves showing relation of concentration of sugar in non-oxygenated medium to amount of carbohydrate stored by yeast.

incubating a fixed quantity of yeast in a sugar solution the amount of carbohydrate stored depended chiefly on the concentration of sugar in the solution in which the yeast had been incubated. The divergences between the three sugars were most apparent in the very dilute solutions; it was here that the amount of carbohydrate present in the original sample of yeast appeared to exercise some influence, for if equal weights of a yeast with a high and a yeast

[1] The mean of three experiments gave 1·6 g. maltose; as one of these values was abnormally low the number 1·7 has been used in the diagram (cp. Table II).

with a low carbohydrate content were incubated in a 0·5 % or in a 1 % solution of the same sugar, the final carbohydrate content was generally higher in

Curves showing relation between increase of fat after incubation in sugar solutions and concentration of solution.

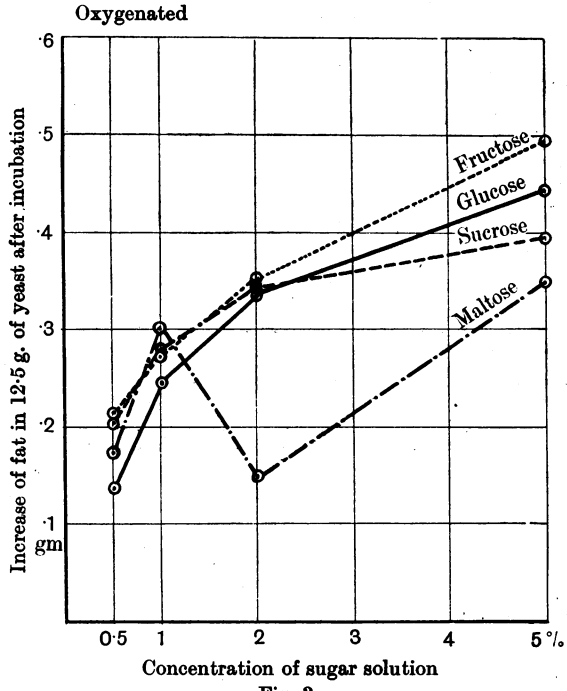

Fig. 3.

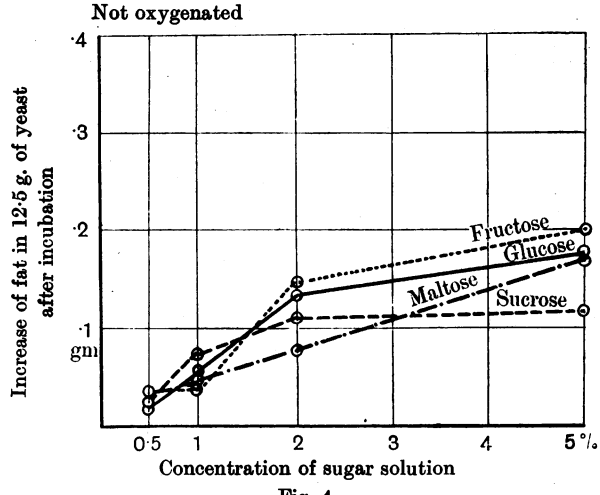

Fig. 4.

the yeast which had the greater content originally. But even with very considerable differences in the carbohydrate contents of the original samples

of yeast, the final amounts of carbohydrate present fell within comparatively narrow limits for any given concentration of sugar.

As the concentration of the solution was increased from 0·5 to 5·0 % the amount of stored carbohydrate rose more sharply at first from 0·5 to 2 %, more gradually from 2 to 5 %. This is apparent from a survey of the curves shown in Figs. 1 and 2.

Fructose in concentrations of from 0·5 to 2 % seems to produce a rather greater storage of carbohydrate than either glucose or sucrose. On the whole we are inclined to think that the figures obtained may possibly represent a real difference in the action of these sugars, though the difference is a small one; the carbohydrate content of yeast after incubation in fructose solutions is higher than that of yeast incubated in glucose and sucrose solutions both with and without oxygenation; and in both cases the difference disappears when the concentration of the sugar rises to 5 %. The effect of oxygenating the sugar solution in which the yeast is incubated is slightly to reduce the carbohydrate content. This result is not invariable but occurs in the majority of cases.

The increase of fat stored up after incubation in an oxygenated solution of glucose, fructose or sucrose is practically the same (Figs. 3 and 4). Fructose gives slightly more fat, but the differences are so small that it is difficult to say whether any significance should be attached to them. The fat-forming power of yeast seems to vary considerably in the different samples and the numbers denoting the amounts of fat stored for any given concentration of sugar in the medium are not as constant as the corresponding numbers denoting the amounts of carbohydrate. For instance four or five experiments were carried out with 5 % solutions of the three sugars; the mean value for the fat content of the yeast after incubation in the fructose solution (oxygenated) was quite appreciably higher than that from the 5 % oxygenated solutions of either glucose or sucrose, but the highest value in any of the experiments was in one of the glucose solutions. We think therefore that though the numbers obtained are suggestive we cannot say with certainty that there is any pronounced difference in the fat-forming powers of these three sugars. With all three the amount of fat deposited in the yeast which has been incubated in a solution of the sugar varies with the concentration of sugar in the medium; there is a gradual flattening in the curve showing the relation of the increase of fat stored to the concentration of sugar, the rate of increase of the fat falling off as the concentration of sugar rises.

The effect of oxygenating the solution is very marked; after oxygenation the fat content is about double as much as in a yeast grown in a similar solution without oxygenation.

The most marked difference between the conditions affecting the storage of fat and of carbohydrate is that, while oxygenation of the solution in which the yeast is incubated diminishes very slightly the amount of carbohydrate in the yeast, it largely increases the formation of fat.

Maltose. Comparison of maltose with the sugars whose action has already been described shows that solutions of maltose of 1 % and over produce markedly greater storage of carbohydrate than do solutions of fructose, glucose or sucrose of similar strength.

Rose [1910] working with *Endomyces vernalis* showed that maltose was a better source of carbon than glucose in a medium which contained also magnesium sulphate, potassium phosphate and asparagine. Lindner and Saito [1910] tested the assimilating power of a very large number of yeasts for various sugars and came to the conclusion that maltose was the best assimilated; Kita's [1914] experiments also confirm this view. Kluyver [1913], starting from the standpoint that these results are not in accordance with the view that all substances before absorption are broken up into the simplest "bricks," repeated Lindner's experiments weighing his yeast after drying at 105° and found definite increases when Kahlbaum's maltose was used, smaller or no increases when the maltose had been first purified by recrystallisation from 80 % alcohol. He found also that the original Kahlbaum sample of maltose contained 0·22 % nitrogen which was reduced after purification to only 0·04 %; he therefore argued that the asparagine of the original solution provided an insufficient nitrogen supply and the effect of the maltose was due to the nitrogenous impurities it contained. Our experiments differed from Lindner's in that our media contained no nitrogen and we measured directly the carbohydrate stored; determinations of the nitrogen in the yeast samples were made both before and after the incubation, and within the limits of the experimental error the nitrogen content was unchanged. The nitrogen contents of the sucrose and maltose used in these experiments were identical. The experiments now carried out seem to us to show quite conclusively that *when yeast is incubated in maltose solutions of from* 1 *to* 5 % *there is much greater carbohydrate storage in the yeast cells than when solutions of glucose, fructose or sucrose are used.*

Maltose solutions however show themselves on the whole to be rather less effective in producing an increase of fat than are solutions of either glucose, fructose or sucrose.

Another point observed while carrying out these experiments is perhaps worthy of notice. After yeast had been incubated in 2 % and in 5 % sucrose solution the weight of the pressed yeast was very much smaller than in the case of yeast from the corresponding solutions of any other sugar. The yeast seemed to have undergone a certain amount of plasmolysis, the dry weight of the yeast, and the amounts of protein, carbohydrate and fat being the same, but the weight of the pressed yeast being only about two-thirds that of the yeast after incubation in solutions of the other sugars.

As the different samples of yeast showed much variation in their power of forming fat, equal quantities of the same sample of yeast were incubated at the same time under similar conditions in a 2 % solution of glucose and in a 2 % solution of one of the sugars, fructose, sucrose or maltose, so that a direct

Table II.

Sugar %	Temp. °C.	Age of yeast. Hours	Original yeast			Incubated in oxygenated solution			Incubated in non-oxygenated solution		
			Fat g.	Carbo-hydrate g.	Protein g.	Fat g.	Carbo-hydrate g.	Protein g.	Fat g.	Carbo-hydrate g.	Protein g.
Glucose:											
0·5	25·5	84	0·1057	1·00	1·36	0·1810	0·55	1·27	0·1262	0·62	1·19
„	Lab.	110	0·1017	1·00	1·39	0·1667	0·57	1·26	0·1040	0·65	1·40
„	24·5	109	0·1012	0·65	1·46	0·2720	0·41	1·25	0·1231	0·42	1·37
„	25	106	0·1040	1·02	1·23	0·1556	0·50	1·12	0·1318	0·66	1·16
„	25	85	0·0646	1·12	1·24	0·3660	0·79	1·15	0·0842	0·78	1·15
1·0	25	110	0·1051	0·82	1·31	0·3358	0·92	1·35	0·1413	0·75	1·28
„	25·5	89	0·1041	1·18	1·26	0·4618	0·83	1·19	0·1830	0·90	1·21
„	24	85	0·0880	1·06	1·14	0·2432	0·71	1·15	0·1457	0·86	1·13
2·0	25·5	89	0·0976	1·07	1·34	0·4220	0·96	1·17	0·2326	0·80	1·27
„	25·5	84	0·1041	1·47	1·18	0·4805	0·84	1·15	0·2350	0·99	1·09
„	25	—	0·0905	1·37	1·15	0·3610	1·03	1·06	0·2220	1·27	1·08
„	25·5	105	0·0805	1·18	1·18	0·3703	0·98	1·17	0·2074	1·22	1·16
„	25	81	0·0785	1·53	1·16	0·4770	1·04	1·11	0·2096	1·04	1·07
„	25	103	0·0890	0·83	1·26	0·4818	0·84	1·24	0·2123	1·17	1·18
„	25	84	—	—	—	0·5095	0·98	1·20	0·2498	0·98	1·20
5·0	27·5	106	0·0985	0·70	1·33	0·5286	1·18	1·31	0·2645	1·20	1·27
„	25·5	84	0·1055	0·69	1·44	0·3956	1·37	1·35	0·2190	1·40	1·29
„	25·5	84	0·9870	1·65	1·13	0·7110	1·08	1·00	0·3495	1·24	0·99
Fructose:											
0·5	25·5	86	0·1018	1·52	1·22	0·1853	0·99	1·19	0·1306	1·01	1·22
„	25	76	0·1042	1·18	1·22	0·4015	0·88	1·21	0·1355	0·99	1·14
„	24·5	84	0·0800	1·46	1·19	0·3477	1·09	1·15	0·1183	1·08	1·15
„	25	88	0·0677	1·03	1·37	0·2380	0·77	1·26	0·1077	0·80	1·26
„	25	85	0·0646	1·12	1·24	0·3404	0·77	1·19	0·0993	0·78	1·21
1·0	25	82	0·1056	1·13	1·22	0·3222	0·85	1·16	0·1248	1·00	1·17
„	25·5	—	0·0925	1·17	1·26	0·4245	1·21	1·23	0·1414	1·08	1·14
2·0	24·5	82	0·1037	1·09	1·31	0·3658	0·97	1·20	0·2327	1·22	1·13
„	24·5	82	0·1037	1·09	1·31	0·4258	1·00	1·25	0·2066	1·22	1·10
„	25	83	0·0885	1·26	1·24	0·5465	1·08	1·14	0·2574	1·18	1·10
„	25	82	0·1056	1·13	1·22	0·4956	0·91	1·17	0·2305	1·08	1·15
„	25·5	—	0·0890	0·83	1·28	0·4729	0·93	1·23	0·3007	1·12	1·23
„	25	80	0·0848	1·45	1·18	0·3794	1·12	1·13	0·2395	1·34	1·17
5·0	25·5	86	0·0979	1·00	1·28	0·5575	1·13	1·49	0·2574	1·24	1·45
„	„	118	0·0977	0·95	1·10	0·6266	1·35	1·07	0·3358	1·35	1·01
„	„	96	0·1046	0·82	1·43	0·5360	1·29	1·37	0·2254	1·10	1·36
Sucrose:											
0·5	25	84	0·0941	1·31	1·23	0·3457	0·68	1·14	0·1252	0·80	1·22
„	„	87	0·0910	1·24	1·25	0·2874	0·77	1·18	0·1226	0·87	1·15
„	„	82	0·0835	0·84	1·33	0·2516	0·73	1·15	0·0891	0·80	1·17
1·0	„	106	0·1022	1·22	1·29	0·2810	0·96	1·21	0·1462	0·99	1·20
„	„	86	0·0970	1·47	1·20	0·4405	0·71	1·18	0·1927	0·86	1·15
„	„	91	0·0706	0·77	1·37	0·3860	0·83	1·22	0·1457	0·82	1·23
2·0	„	106	0·1050	1·13	1·30	0·4355	1·03	1·27	0·2107	1·12	1·23
„	„	81	0·0785	1·53	1·16	0·4704	0·80	1·09	0·1996	0·93	1·09
„	„	84	0·0943	1·12	1·23	0·3980	0·90	1·16	0·2012	0·99	1·16
5·0	„	84	0·0815	1·23	1·25	0·3803	1·18	1·13	0·2037	1·40	1·15
„	„	85	0·0998	1·25	1·30	0·3500	1·30	1·16	0·1750	1·40	1·15
„	„	106	0·0928	1·00	1·24	0·6008	1·22	1·10	0·2522	1·18	1·10
„	„	96	0·0888	1·10	1·28	0·6097	1·07	1·18	0·2398	1·06	1·12
Maltose:											
0·5	25·5	87	0·1040	1·00	1·30	0·3041	0·71	1·24	0·1350	0·77	1·25
„	24·5	85	0·0867	1·02	1·32	0·2433	0·72	1·30	0·0935	0·72	1·21
1·0	25·5	110	0·1001	1·00	1·25	0·2978	1·19	1·26	0·1591	1·17	1·17
„	24·5	96	0·0673	0·72	1·38	0·4919	0·79	1·33	0·0972	0·68	1·27
2·0	25.	84	0·0870	0·86	1·18	0·2134	1·31	1·09	0·1781	1·55	1·23
„	25·5	105	0·0805	1·18	1·18	0·2962	1·33	1·27	0·1880	1·61	1·18
„	24	108	0·0878	0·93	1·33	0·1863	0·98	1·27	0·1238	1·33	1·24
5·0	26	104	0·1045	1·05	1·30	0·4205	1·82	1·21	0·2760	1·71	1·18
„	25	118	0·0977	0·95	1·10	0·4697	1·95	1·02	0·3165	1·80	1·05
„	25·5	85	0·0945	1·29	1·25	0·4639	1·76	1·23	0·2328	1·30	1·14

comparison might be made. The fat contents of the yeasts incubated in the oxygenated solutions of glucose, fructose and sucrose were found to agree within limits of experimental error, but there was appreciably less fat in the yeast incubated in the maltose solution.

Table III. *Showing increase of fat and carbohydrate produced by incubating equal quantities of the same samples of yeast in* 2 % *solutions of two different sugars.*

			Original yeast		Incubated in oxygenated solution		Incubated in non-oxygenated solution	
Sugar 2 %	Temp. ° C.	Age of yeast. Hours	Fat g.	Carbo-hydrate g.	Fat g.	Carbo-hydrate g.	Fat g.	Carbo-hydrate g.
Glucose	25	103	0·0890	0·83	0·4818	0·84	0·2123	1·17
Fructose	,,	—	—	—	0·4729	0·93	0·3007	1·12
Glucose	,,	81	0·0785	1·53	0·4770	1·04	0·2096	1·04
Sucrose	,,	—	—	—	0·4704	0·80	0·1996	0·93
Glucose	,,	105	0·0805	1·18	0·3703	0·98	0·2074	1·22
Maltose	,,	—	—	—	0·2962	1·33	0·1880	1·61

THE PART PLAYED BY OXYGEN IN PRODUCING AN INCREASE OF FAT.

In order to be sure that the effect of the oxygen was not merely the mechanical one of removing the carbon dioxide, such as that described by Slator [1921], we replaced the current of oxygen by one of hydrogen when incubating the yeast in a 5 % solution of glucose. The control in which no gas was passed gave figures similar to those obtained when a current of hydrogen was passed through the solution. It follows therefore that the effect of the oxygen is not a mechanical one but is specific to oxygen.

PART III. THE INFLUENCE OF PHOSPHATES.

The formation of hexosephosphate when sugars are fermented by yeast juice in the presence of phosphates was first established by Harden and Young [1905, 1908, 1909]; this compound appears to be fructose diphosphate and it is formed when either glucose, mannose or fructose undergoes fermentation. Whereas Harden and Young regard the formation of this compound as an essential reaction in the fermentation of sugar brought about by the living yeast cell, Neuberg, Levite and Schwenck regard its formation as a pathological phenomenon [1917]. Since its discovery a large amount of work has been carried out on the constitution and properties of this substance and evidence has been brought forward suggesting that the presence of a hexosephosphate may not be confined to yeast juice [Embden and Lacquer, 1921]. The probable presence of this substance in muscle [Embden and Lacquer, 1914, 1921] and the detection of an enzyme acting on it in ossifying cartilage [Robison, 1923] are also observations of great interest.

It is well known that the radicals of phosphoric acid and of the fatty acids are associated together in the lipins; the wide distribution of these compounds throughout both the animal and vegetable kingdoms has given rise to much speculation as to their function in the metabolism of the cell.

They have been regarded as the form in which fat is transported in the body, and evidence has been brought forward that in a lactating animal the phosphatide content of the blood in the mammary vein is less than that in the jugular vein [Meigs, Blatherwick and Cary, 1919, 1920]: from this the conclusion has been drawn that the phosphatides of the blood are utilised by the mammary gland to form milk fat. In spite however of the large number of investigations which have been carried out, the function of the lipins and the rôle they play in the life of the cell cannot be said to have been satisfactorily elucidated.

The fact that the hexose molecule and the fatty acid radicals are each found in association with the phosphate radical suggests that a more complete knowledge of the phosphorus metabolism of the cell may throw some light on the processes by which fat is manufactured from carbohydrate in the living organism. We endeavoured therefore to find out whether the formation of fat in the yeast cell was in any way influenced by the presence of phosphates in the solutions in which the yeast was incubated.

The first question investigated was whether the conditions under which the yeast was incubated could be so modified as to produce an increased storage of phosphate in the yeast cell. We therefore examined the effect of changes in the composition of the medium in which the yeast was incubated and determined the effect on the total phosphorus content of the yeast produced by the addition of sugar to the phosphate solution in which the yeast was incubated.

The method adopted was a follows: 12·5 g. of pressed yeast which had been well washed were incubated for 45 hours at 25° in a litre of a solution of sugar containing a mixture of 0·3962 % Na_2HPO_4 and 0·0286 % KH_2PO_4, a mixture giving a p_H of about 7·8. The sugar to be investigated was then added in varying concentrations, the percentage of phosphate being kept constant throughout the series of experiments. At the end of 45 hours the yeast was filtered off, washed until the washings gave no reaction for phosphate and the total amount of phosphorus it contained determined by Neumann's method. An analysis of 12·5 g. of the original sample of yeast was also made so that the increase of phosphorus could be calculated. The fat and carbohydrate were estimated as previously described [Smedley MacLean, 1922] after hydrolysing the yeast by boiling for two hours with normal acid; the protein content of the yeast was also determined.

It was found that the increased amount of phosphate taken up by the yeast cell depended on the concentration of sugar in the solution in which the yeast had been incubated.

The effect of the three sugars, glucose, fructose and sucrose, was practically identical. The amount of phosphate taken up from the solution by the yeast cell varied with the concentration of sugar, increasing as the proportion of sugar in the medium rose from 0·5 to 5·0 %. The action of maltose also produced an increase in the phosphorus content of the yeast after incubation,

the increase being dependent on the strength of the maltose solution in which the yeast was incubated. The increase of phosphate in the yeast was however appreciably less when maltose was substituted for the other sugars mentioned, the difference being most marked in the stronger solutions.

The actual numbers obtained are shown in Table IV and the relation of the increase of phosphate to the concentration of the sugar solution is expressed by the curves shown in Figs. 5 and 6.

Table IV. *Showing amount of phosphorus in* 12·5 *g. of pressed yeast after being incubated for* 45 *hours in a litre of sugar solution containing* 0·3962 % Na_2HPO_4 + 0·0286 % KH_2PO_4.

	Amount of P in sample				Amount of P in sample		
Conc. of sugar %	(1) Original g.	(2) After incubation in oxygenated solution g.	(3) After incubation without oxygenation g.	Conc. of sugar %	(1) Original g.	(2) After incubation in oxygenated solution g.	(3) After incubation without oxygenation g.
Glucose:				Fructose:			
0·5	0·0613	0·0687	0·0663	0·5	0·0561	0·0777	0·0568
	0·0617	0·0689	—		0·0583	—	0·0586
1	0·0618	0·0754	0·0734		0·0679	0·0716	0·0656
	0·0642	0·0764	0·0731		0·0673	0·0713	0·0650
	0·0602	0·0865	0·0893	1	0·056	0·067	0·0697
	0·0598	0·0850	0·0870		0·057	0·062	—
2	0·0611	0·0860	0·0786	2	0·0682	0·1012	0·0900
	0·0586	0·0870	—		0·0718	0·1023	0·0921
	0·0638	—	0·0740		0·0598	0·0880	0·0747
	—	—	0·0730		0·0566	0·0879	0·0757
					0·0611	0·0860	0·0761
4	0·0650	0·1050	0·1024		0·0586	0·0846	0·0742
	0·0633	0·0989	0·0882				
5	0·0607	0·1021	0·1035				
	0·0609	—	0·1124	5	0·077	0·128	0·102
	0·0580	0·1252	0·1158		0·065	0·123	0·098
	0·0586	0·1310	0·1140				
	0·0694	0·1023	0·0962				
	0·0694	0·1009	0·0919				
Maltose:				Sucrose:			
0·5	0·0640	0·0566	0·0681	0·5	0·0606	0·0668	0·0639
	0·0637	0·0510	0·0653		0·0578	0·0749	0·0634
1	0·0634	0·0770	0·0602	1	0·0620	0·0706	0·0632
	0·0630	0·0706	0·0611		0·0618	0·0717	0·0676
2	0·0600	0·0776	0·0700	2	0·0708	0·0934	0·0839
	0·0594	—	0·0714		0·0742	0·0965	0·0799
5	0·0656	0·0949	0·0767	5	0·0596	0·1102	0·0883
	0·0684	—	0·0757		0·0603	0·1105	0·0881
	0·0709	0·0903	0·0772				
	0·0679	0·0898	0·0780				

It is of course possible that the numbers obtained at the end of the experiment represent a balance; that for instance hexosephosphate is being continually formed and decomposed and that in the dilute sugar solutions the figures are low because there is insufficient sugar to reform the hexosephosphate which has been decomposed. The residual sugar was always determined at the end of each experiment; it was generally found that in the

0·5 % solutions all the sugar had been used up, but in the higher concentrations some sugar always remained.

The effect of oxygenation on the amount of phosphate taken up by the yeast cell was found to be quite marked. Oxygenation of the solution increased considerably the amount of phosphate stored by the yeast cell.

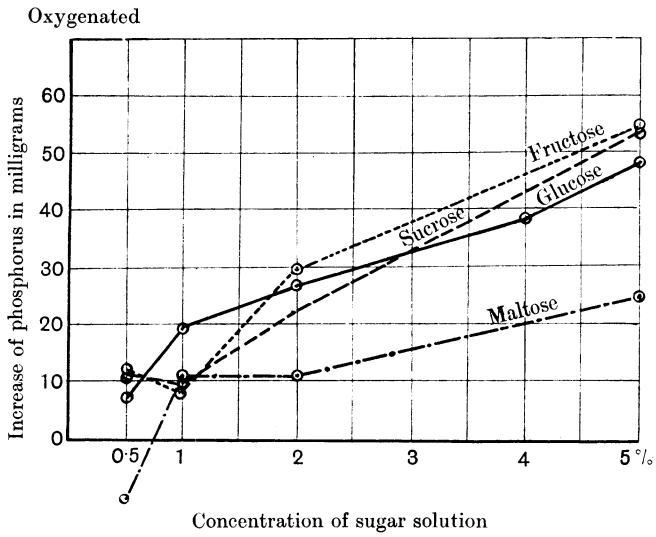

Fig. 5. Curves showing relation between concentration of sugar in solution and increase of phosphorus in yeast.

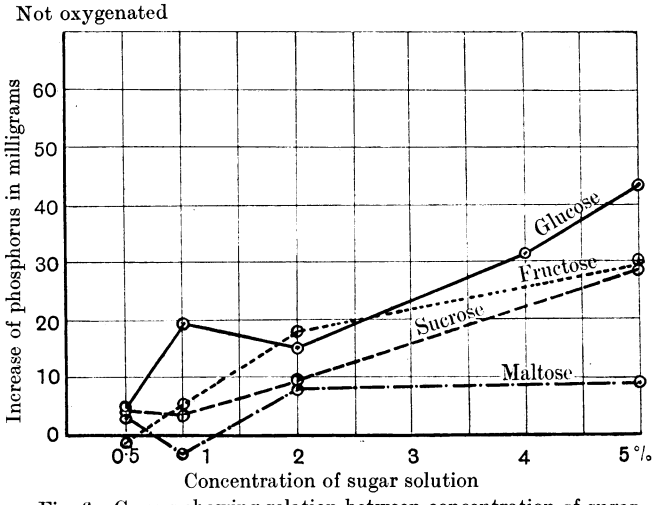

Fig. 6. Curves showing relation between concentration of sugar in solution and increase of phosphorus in yeast.

The above results indicate that phosphate is taken up by the yeast cell from the medium in which it is incubated, in association with sugar and strongly support Harden's view that hexosephosphate is a normal stage in the metabolism of the cell.

The second point investigated was whether the addition of phosphate to the sugar solution in which the yeast was incubated had any influence on the amount of fat or carbohydrate contained in the yeast.

The method by which this was determined was to incubate quantities of 12·5 g. of the same sample of yeast under precisely similar conditions in a litre of sterilised sugar solution with and without the addition of phosphate and to analyse the sample of yeast before and after it had been incubated. Four Winchester quart bottles were placed in the incubator at the same time; two contained a litre of the sugar solution which it was desired to investigate, and in each of the other two was placed a litre of the sugar solution to which the phosphate mixture had been added in the proportions above described.

The solution in one of each pair of bottles was oxygenated throughout the experiment so that the effect of oxygenating the solution could at the same time be determined.

Influence of the addition of phosphates on the formation of fat.

The results of these experiments show that the presence of phosphates exerts a quite considerable effect in increasing the storage of fat if the solution in which the yeast is incubated is oxygenated during the experiment. If the solution be not so oxygenated the result is less marked, but in the majority of cases even when the medium is not oxygenated the yeast in the phosphate-containing solution has the higher fat content. Very dilute solutions of fructose were rather variable in their behaviour, but with stronger solutions the yeast always showed an increased fat content after incubation. The differences in the increase of fat produced by the addition of phosphates to the oxygenated solutions of the different sugars examined were of the same order; no differences which could be regarded as significant were observed between the effects of maltose, glucose, fructose or sucrose.

Analysis of the yeast samples before and after incubation clearly indicated that the addition of phosphates to the oxygenated sugar solutions produced an increased fat content of the yeast; we were however unable to establish a relation between the amount of increase of fat produced by the addition of phosphates to the oxygenated solution and the concentration of sugar in the medium.

Influence of the addition of phosphates on the carbohydrate content.

We have shown that oxygenation of the sugar solution in which the yeast is incubated on the whole tends to diminish the carbohydrate content of the yeast when compared with yeast grown in a similar solution without oxygenation; this effect is however not invariable and in most cases was found to be slight.

The effect of the addition of phosphates to the sugar solution was somewhat to increase the difference between the carbohydrate content of the

Table V. *Showing the effect of incubating yeast in solutions of* 0·4 % *alkaline phosphate with different concentrations of sugar. Influence on fat and carbohydrate content.*

			(1) Original sample of 12·5 g. pressed yeast			(2) After incubation in sugar solution oxygenated			(3) After incubation in sugar solution not oxygenated			(4) After incubation in sugar solution + phosphate solution oxygenated			(5) After incubation in sugar solution + phosphate solution not oxygenated		
%	Temp. °C.	Age of yeast. Hours	Fat g.	Carbohydrate g.	Protein g.	Fat g.	Carbohydrate g.	Protein g.	Fat g.	Carbohydrate g.	Protein g.	Fat g.	Carbohydrate g.	Protein g.	Fat g.	Carbohydrate g.	Protein g.
Glucose:																	
0·5	25	106	0·1040	1·02	1·23	0·1556	0·50	1·12	0·1318	0·66	1·16	0·2257	0·63	1·14	0·1198	0·75	1·07
1	24	85	0·0880	1·06	1·14	0·2432	0·71	1·15	0·1457	0·86	1·13	0·4335	0·70	1·16	0·1666	0·99	1·16
2	25	—	0·0905	1·37	1·15	0·3610	1·03	1·06	0·2220	1·27	1·09	0·4340	0·78	1·07	0·2580	1·02	1·09
	25	94	0·0854	1·17	1·27	—	—	—	—	—	—	0·5389	0·98	1·19	0·2615	0·99	1·17
4	25	79	0·0772	0·89	1·27	0·2690	1·03	1·19	0·2470	1·17	1·20	0·4528	0·91	1·24	0·2391	1·02	1·22
5	25·5	87	0·1029	0·81	1·30	0·3215	1·18	1·27	0·2360	1·26	1·19	0·4522	0·87	1·22	0·2544	1·06	1·16
	25·5	109	0·1102	1·02	1·26	—	—	—	—	—	—	0·9012	1·26	1·16	0·4542	1·62	1·11
Fructose:																	
0·5	24·5	84	0·0800	1·46	1·19	0·3477	1·09	1·15	0·1183	1·08	1·15	0·3089	0·91	1·15	0·1089	1·13	1·15
	25	88	0·0677	1·03	1·37	0·2380	0·77	1·26	0·1077	0·80	1·26	0·3231	0·75	1·24	0·1270	0·74	1·25
1	25·5	—	0·0925	1·17	1·26	0·4245	1·21	1·23	0·1414	1·08	1·14	0·3304	0·87	1·30	0·1464	0·92	1·26
2	25	83	0·0885	1·26	1·24	0·5465	1·08	1·14	0·2574	1·18	1·10	0·5495	1·05	1·18	0·1822	1·36	1·12
		80	0·0848	1·45	1·18	0·3794	1·12	1·13	0·2395	1·34	1·17	0·5550	1·06	1·17	0·2524	1·19	1·14
		94	0·0854	1·17	1·27	—	—	—	—	—	—	0·6167	1·06	1·18	0·2615	1·28	1·18
5	25·5	—	0·1046	0·82	1·43	0·5360	1·29	1·37	0·2254	1·10	1·36	0·8180	1·25	1·33	0·3680	1·63	1·35
Sucrose:																	
0·5	25	82	0·0835	0·84	1·33	0·2516	0·73	1·15	0·0891	0·80	1·17	0·3334	0·72	1·17	0·1242	0·79	1·01
1	27·5	91	0·0706	0·77	1·37	0·3860	0·83	1·22	0·1457	0·82	1·23	0·3562	0·84	1·24	0·1277	0·83	—
2	25	84	0·0943	1·12	1·23	0·3980	0·90	1·16	0·2012	0·99	1·16	0·5441	0·94	1·13	0·2021	1·12	1·14
5	25	96	0·0888	1·10	1·28	0·6097	1·07	1·18	0·2398	1·06	1·12	0·7390	0·94	1·15	0·2669	1·17	1·15
Maltose:																	
0·5	24·5	85	0·0867	1·02	1·32	0·2433	0·72	1·30	0·0935	0·72	1·21	0·3190	0·71	1·23	0·0818	0·46	1·25
1	24·5	96	0·0673	0·72	1·38	0·4919	0·79	1·33	0·0972	0·68	1·27	0·4960	0·81	1·29	0·1128	0·58	1·31
2	25	84	0·0870	0·86	1·18	0·2134	1·31	1·09	0·1781	1·55	1·23	0·3552	1·14	1·06	0·2083	1·22	1·16
	24	108	0·0878	0·93	1·33	0·1863	0·98	1·27	0·1238	1·33	1·24	0·3454	0·70	1·26	0·1411	0·92	1·26
5	25·5	85	0·0945	1·29	1·25	0·4639	1·76	1·23	0·2328	1·30	1·14	0·5608	1·62	1·17	0·2335	1·48	1·03
	25	96	0·1068	0·90	1·33	—	—	—	—	—	—	0·4907	1·48	1·25	0·2309	1·89	1·26

yeast incubated in the sugar solution with and without the passage of a current of oxygen. The carbohydrate content of the yeast incubated in the non-oxygenated solution was the greater and the addition of phosphate to the sugar solutions which were oxygenated during the period of incubation led to a distinct diminution in the carbohydrate content of the yeast.

Influence of the addition of phosphates on the protein content.

The differences in the protein content of the yeast before and after incubation in the sugar solutions to which phosphates had been added were very small; and considering the difficulties of carrying out these experiments without any loss of yeast during filtration, they cannot be regarded as outside the range of experimental error.

Summary and discussion of results.

(1) When yeast was incubated in oxygenated water, part of the carbohydrate originally present disappeared and an increase of fat took place. The addition of propyl, butyl and *iso*-amyl alcohols in decimolar concentration exerted an inhibitory effect; decimolar solutions of the sodium salts of formic, propionic and butyric acids, glycol, glycerol and acetone behaved like water.

(2) When yeast was incubated in oxygenated 0·5 % solutions of ethyl alcohol or of the sodium salts of acetic, lactic or pyruvic acids, an effect was exerted on the fat content of the yeast similar to that produced by 0·5 % solution of glucose. Increasing the concentration of these substances in the solutions did not however lead to further increase of the fat content of the yeast, as in the case of the sugars.

A possible explanation is that these simpler molecules may be used to build up carbohydrate and only indirectly lead to the production of fat; this suggestion is supported by the observation that in certain cases where the yeast was incubated in oxygenated solutions of ethyl alcohol and of sodium acetate an increase in the total carbohydrate of the yeast cell was observed.

(3) When yeast was incubated under the conditions described in a solution of fructose, glucose or sucrose, at the end of 45 hours both fat and carbohydrate were found to have been stored up by the yeast cells. The amounts stored up depend on the concentration of sugar in the medium and are independent of the nature of the sugar; the rate of increase of the carbohydrate content diminishes as the concentration of the sugar in the medium rises; the rate of increase of the fat content also falls, but much more than that of the carbohydrate content, as the sugar concentration increases.

(4) Maltose differs markedly in its behaviour from the three sugars, glucose, fructose and sucrose. It is more potent in producing a storage of carbohydrate and rather less effective in building up fat.

The difference in the power of storing carbohydrate is so marked that it seems impossible that the maltose can be split into glucose before assimilation, and we must conclude that maltose is directly dealt with as such by the yeast

cell and there built up into reserve carbohydrate. It is interesting to recall that the recent work of Irvine [1923] points to the presence of a maltose unit in the glycogen molecule; this conclusion is in accordance with the behaviour of the yeast cell which stores carbohydrate more readily when fed with a solution of maltose than with one of glucose.

The storage of fat after yeast has been incubated in maltose solutions was rather less marked than with the other sugars; in 0·5 and 1 % oxygenated solutions as much fat was formed by the yeast as when it was incubated in the corresponding solutions of glucose, fructose or sucrose, but after incubation in a 2 or 5 % maltose solution less fat was formed. No increase in fat content corresponding with the increased carbohydrate content was observed.

(5) Oxygenation of the medium throughout the period of incubation made a very great difference to the amount of fat produced, but made little difference to the final carbohydrate content, generally producing a slight diminution in amount.

(6) The addition of phosphates to the oxygenated sugar solutions produced an increase in the amount of fat stored and a diminution in the amount of carbohydrate. If the solutions were not oxygenated, the increase of fat was small or absent.

(7) If about 0·4 % of alkali phosphates was added to solutions of the different sugars in which the samples of yeast were incubated, phosphate was taken up by the yeast cell in proportion to the concentration of sugar in the solution in which the yeast was incubated. The amount of phosphate was greater in the yeast incubated in the oxygenated solutions than in that incubated in solutions which were not oxygenated.

These results seem best explained by the hypothesis that carbohydrate storage in yeast takes place in two ways: (1) as glycogen or some other similar compound giving a reducing sugar on hydrolysis and (2) as a hexosephosphate which forms the first stage in the transformation of carbohydrate to fat.

The view that carbohydrate is absorbed and combined with phosphate forming a hexosephosphate is supported by the work of Harden and Young on the isolation of hexosephosphates from yeast juice and by the evidence now brought forward that the phosphate content of the yeast depends on the concentration of the sugar in the medium in which the yeast is incubated.

The second part of the hypothesis now put forward that the formation of a hexosephosphate may be regarded as the first stage in the transformation of carbohydrate to fat is supported by the fact that the addition of phosphates to the solution of sugar in which the yeast is incubated somewhat increases the fat content of the yeast if the solution be oxygenated during the period of incubation.

Although the proportion of oxygen is much less in the fat than in the carbohydrate molecule, the part played by the oxygen is evidently a very important one in the transformation of carbohydrate to fat. Oxygenation of

the sugar solution in which yeast is incubated produces a yeast with more than double the fat content of a yeast incubated in a sugar solution which has not been so oxygenated; and if additional phosphate be added to the medium the fat content is still further raised if the solution continues to be oxygenated.

The increase of fat stored up when phosphate is added to the oxygenated sugar solution in which the yeast is incubated seems to us to be best explained by assuming that oxidation of a hexosephosphate or of some material derived from it forms an essential stage in the story of the fat metabolism of yeast.

It is possible that simpler molecules such as those of pyruvic acid or aldehyde may be formed at a later stage, or it is possible that groups of this nature may be associated in some complex compound containing the phosphate radical. At present there is no evidence which throws light on this question and it remains only a matter of speculation.

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